SOME GENERAL FEATURES OF THE METAMORPHOSIS OF THE FLAG WEEVIL MONONYCHUS VULPECULUS FABR.

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I HAVE been for some time desirous of studying the development of some beetle which would represent metamorphosis in as complete a condition as is found within the order Coleoptera. Last summer I found an abundance of the flag weevil (*Mononychus vulpeculus* Fabr.) in all stages; and this furnished me the opportunity for which I waited. The larvae of this beetle are little fat grubs, which eat the seeds of the blue flag (*Iris versicolor* Linn.). They are sheltered from first to last within the flag capsule and are very degenerate. They lack eyes, antennae, and legs, as well as wings. They represent a sort of ecological specialization, common among the higher insects, manifest in the adaptation of life to very special situations, and of life history to conditions of transient food supply.

I. *Life History.*

The life history of this long familiar species seems not to have been fully made known.[1] While gathering my material I was not seeking to determine the full life history, but now I find that my collections and notes reveal it pretty completely. Collected material gathered in at intervals of two or three days give data as follows: Eggs were first found June 8. The beetles had just begun to oviposit on the earliest of the flag flowers, first opened that day. Larvae were first found June 29, at which

[1] Dr. John Hamilton published fragmentary notes on its life history in 1894 ("Mononychus vulpeculus and its Parasites," *Entom. News*, vol. v, pp. 287, 288), describing oviposition and the form and feeding habits of the larva, and citing an instance of great destructiveness on the part of two parasites, *Pimpla inquisitor* Say and *P. pterelas* Say.

time but few eggs were hatched. Pupae were first found August 5, and newly transformed imagoes, August 8, two months after egg-laying began.

Examining my collection of several hundred larvae, after the manner of lepidopterists, I find among them three sizes of head, so distinct as to certainly indicate three larval stages. The first, which is of the size attained before hatching, measures in diameter .24–.26 mm., the second .40–.44 mm., and the third .81–.85 mm.

My notes and collection labels together indicate the following life history:

1. An egg stage, lasting about three weeks. The eggs lie at the bottom of punctures made through the wall of the flag ovary by the mother-beetle with her rostrum. The egg is pellucid white, broadly oblong-oval in outline, and measures .38 by .70 mm.

2. A first larval stage, lasting about five days. At the end of this stage an average larva measures 2.2 mm. in length by .4 mm. in greatest diameter.

3. A second larval stage, lasting perhaps ten days (certainly not over two weeks), at the end of which the larva measures 4.60 mm. in length by 1.02 mm. in greatest diameter. Thus far the larva remains slender and quite elongate. During these two stages it traverses the outer face of from three to five seeds, leaving a slowly widening, shallow, brown furrow across their surfaces.

4. A third larval stage, lasting a very little more than two weeks, and divided into two periods:

(*a*) A period of feeding, and extraordinarily rapid growth, lasting hardly more than a week. The greater part of increase in size is attained during this short period. During it the larva is boring through the center of several seeds, feeding on their highly nutritive endosperm. At the end of it the larva measures 6.5 mm. by 2.5 mm.

(*b*) A period of transformation to the pupa.

5. A pupal stage, lasting, apparently, not more than a week, spent within the larval burrow. The pupa is naked and smooth, except for a pair of recurved spines on the tip of the abdomen.

6. A period of adult life, lasting ten or more months. Of this time a month or more is spent (lasting until the bursting of the

flag capsules in autumn) quietly within the larval burrow; eight or more months are spent in hiding in winter quarters; activity only begins with the season of iris flowering, and lasts for about a month. Oviposition continues sparingly after the first week. Except at the beginning of the season, several developmental stages may be taken at the same time, and this fact renders dates of *first observation only* of value as indices of life history.

The more striking features of this life history are:

1. The small number of larval stages, for a representative of this order.[1]

2. The exceedingly rapid growth during the first period of the third larval stage. That an animal which will live a year should attain the greater part of its growth within a week is indeed a striking phenomenon. To be sure, this growth is mainly increase of fat.

3. The long period of adult inactivity, extending through two stretches of warm weather.

The metamorphosis of this beetle is very complete. The segregation of the development life into growth period (period of partial anabolism — fat-making) and differentiation period is very marked. The transformation of the degenerate larva, lacking wings, legs, antennae, eyes, optic lobes, and salivary glands, into the adult with all these parts well developed, is very rapid. There are excellent reasons for believing that these things have been independently acquired in the order Coleoptera. Internal metamorphosis has as yet been studied only in such representatives of this order as, in the larval stages, have legs and antennae and eyes, and undergo a metamorphosis much less rapid and complete. Therefore, it should be important to learn whether this increasing periodicity in life history has produced the same changes here as in other orders, whether disappearance of larval appendages has resulted in the internal development of imaginal discs, whether rapid metamorphosis is accompanied by phagocytosis, etc.

The external signs of internal metamorphic processes begin to

[1] Dr. C. V. Riley found four larval stages in the clover leaf weevil. *Vide*, "The Clover Leaf Beetle Phytonomus punctatus Fabr.," *Rept. U. S. Dept. of Agric.* for 1881, pp. 171–179, Pl. X. The Phytonomus larva is less degenerate.

appear almost as soon as the larva is done feeding. There is a slight loosening of the cuticle, and contraction of the body away from it, especially on the dorsal side of the thoracic segments and of the head. The budding legs and wings, already visible through the transparent skin (Fig. 1), begin to grow and extend downward. The fat at the interior end of the body begins to lose its mottled, slightly grayish appearance, and becomes homogeneous, translucent, slightly yellowish-white.[1]

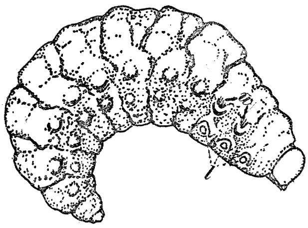

FIG. 1. — Full-grown larva of the flag weevil (*Mononychus vulpeculus* Fabr.). *w*, wing buds, and *l*, leg buds, as seen through the skin.

Then the larval skin is cast, and the pupa appears with all the adult appendages clearly recognizable. After this the progress of internal changes may best be gauged by pigmentation; first, in the eyes, then in the tips of the hind wings, and lastly in the general integument. Some of the corresponding internal phenomena will be discussed under the following headings.

II. *The Hypodermis during Metamorphosis.*

The hypodermis of this weevil is not, so far as observed, destroyed during metamorphosis to be again rebuilt in any part. The cells, however, while maintaining fairly constant relations at their ends, externally with the covering cuticle and internally with the tenuous basement membrane, take on remarkable variety of form and show a fine capacity for shifting, massing, or scattering themselves, previous to the definitive formation of the adult chitin. This might have been anticipated, in view of the exquisite sculpture and ornamentation of the adult beetle. It will be of interest to consider first briefly the origin of the appendage buds ("imaginal discs").

Wings and legs appear at the beginning of the third larval stage, each as a slight thickening of the hypodermis, showing almost from the beginning a slightly concentric arrangement of the elongating cells. Fig. 2, *B*, shows the beginning of a middle

[1] Correspondingly it ceases to be stained black with osmic acid in fixation.

leg. A wing bud would differ only in having the inner surface of the hypodermis free from tracheae, etc.

The striking difference between the behavior of the cells in these buds, and the behavior of hypodermis cells elsewhere (observed, doubtless, by every one who studies sections of insect larvae), I have thought it worth while to emphasize in this figure. Fig. 2, *A*, shows the crumpling which precedes molting everywhere except in these buds. Fig. 2, *B*, is a bud, and shows instead the thickening of the hypodermis and the compression of its cells. Elsewhere the hypodermis stretches as it grows;

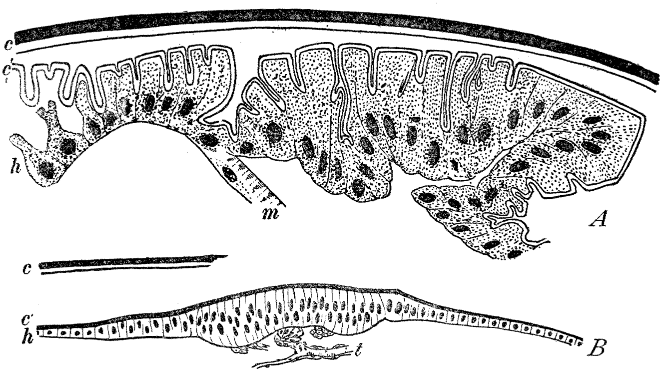

Fig. 2. — Two dispositions of hypodermis. *A*, vertical section of the frons at the end of the second larval stage, showing the crumpling of the hypodermis. *B*, vertical section of the bud of the middle leg at the time of molting at the end of the second larval stage, showing the thickening of the hypodermis, without crumpling. *c*, old chitine; *c'*, new chitine; *m*, developing muscle fiber; *t*, trachea.

the cells separate as they multiply; and that is true also of the hypodermis of the appendages, later, when the time has come for their extension. This we call retarded development, but the physiological explanation of it is still lacking.

There is no invagination of wing and leg buds in this beetle. Even the shallow hypodermal pockets formed about them in the more generalized Coleoptera are absent. The buds do not retreat from the surface. Fig. 1 shows their appearance as seen through the thin integument of the full-grown larva. Fig. 3 is a section of the wing at this time. The inner wall of the projecting shelf of hypodermis below the wing tip is all there is to represent the so-called "peripodal membrane." With

metamorphosis the wing begins to elongate from its apex and soon crowds downward past the shelf; and even before the casting of the last larval skin the general form of the adult elytron with the principal furrows upon its surface will have appeared.[1]

The scales of this weevil are wholly developed during the pupal period. They vary in form from simple sensory hairs as in the antennal club, and slender, lanceolate, sensory scales in the tarsal brushes, to flat, longitudinally fluted, Lepidopter-like scales of yellow and black colors on the dorsal and more exposed surfaces, and delicate, short-plumose, white, or pale yellow scales on the less exposed surfaces. These, one and all, arise from ordinary, single hypodermis cells, after the manner of the development of the scales in the lepidopterous wings, as described by Mayer[2] *et al.*

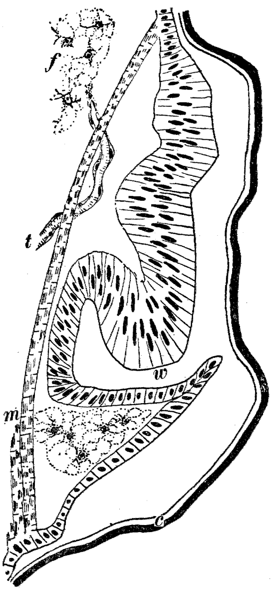

FIG. 3. — Vertical section of the fore wing in a grown larva. *w*, the wing apex; *c*, the loose chitine; *m*, muscle; *t*, trachea; *f*, fat.

First, in early pupal life the cells destined to produce the scales become much larger than their fellows and retreat a little from the surface, so that their nuclei appear at a lower level than the level of the other nuclei. Then each scale mother-cell loses its attachment to the basement membrane, becoming rounded off internally and sometimes acquiring a vacuole, and puts forth a process (the scale that is to be) between the adjacent surface cells (*cf.* Fig. 9). From this process the scale develops, the peculiarities of its own scale kind differentiating rather tardily.[3]

[1] There is no need to recount the wing development, since in all important features it is the same in this beetle as in a Coccinellid of which Professor Comstock and I have hitherto published an account (*Amer. Nat.*, vol. xxxiii, pp. 845–858, 1899).

[2] Mayer, A. G., "The Development of the Wing Scales and their Pigment in Butterflies and Moths," *Bull. Mus. Comp. Zoöl.* Vol. xxix, pp. 209–236, 7 plates, 1896. (Gives bibliography of earlier studies.)

[3] By far the most interesting features of the hypodermis are found in the metamorphosis of the head and the development of the rostrum. These will

III. *The Development of the Legs.*

Aside from a few not very recent accounts of the development of the legs in Diptera, in which the leg buds are deeply invaginated in the larva, Gonin's account of them in the butterfly Pieris remains the only considerable one. And in Pieris there are larval legs, well developed and functional from the first. It will be well, therefore, to notice here some points in the development of the legs of this weevil.

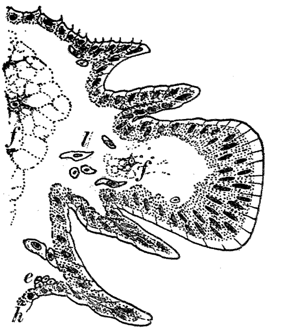

FIG. 4. — Longitudinal section of the middle leg in a grown larva. *f*, fat; *l*, leucocytes; *e*, embryonic cells. (Drawn from a preparation made in my laboratory by Mr. C. Betten.)

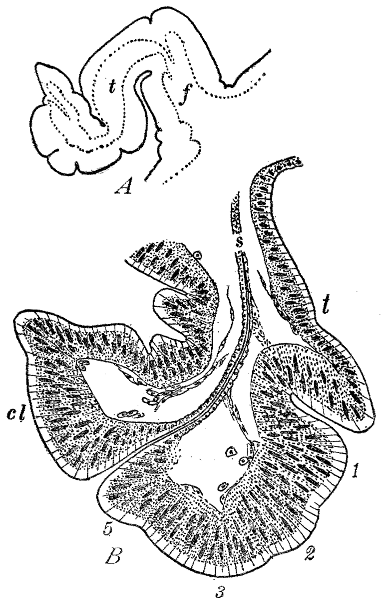

FIG. 5. — The leg of a larva, just before pupation. *A*, the entire leg in outline, and in part in optic section. *B*, a longitudinal section of the tarsus. *f*, femur; *t*, tibia; *1*, *2*, *3*, *5*, tarsal segments; *cl*, claw; *s*, developing tendon.

We have already called attention to Fig. 2, illustrating their origin. Fig. 4 is a longitudinal section of the leg of a grown larva, such as is shown in Fig. 1. Three principal divisions of the leg are already marked out by two deep constrictions. From the time of the beginning of the metamorphosis the growth of the legs is extremely rapid. Fig. 5, *A*, represents one of them as it appears after stripping off the larval skin just before pupation. (Fig. 8 shows wing and leg together, and in their relations to other parts.) The nine segments of the leg are

constitute the subject of another paper, which is now being prepared by a student in my laboratory.

now clearly recognizable, all save one — the fourth segment of the tarsus, whose size is small in the adult, and whose suppression thus seems to extend back into the ontogeny of the species. Fig. 5, *B*, represents a longitudinal section through the tarsus at the same stage. This shows well the condition of the hypodermis at the time when all is ready for that great extension which accompanies pupation. Here is shown also the development of the strong tendon which protracts the claw, as a hypodermal invagination between segment five of the tarsus and the base of

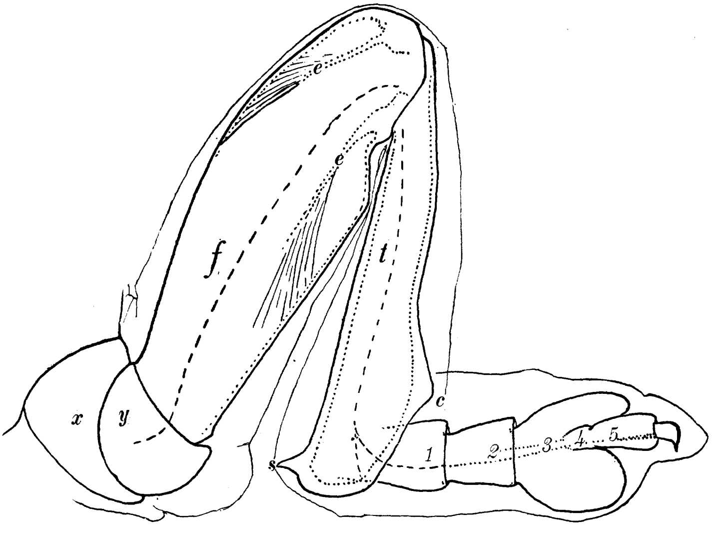

FIG. 6. — Leg of young pupa, within the pupal skin, in part in optic section. *x*, coxa; *y*, trochanter; *f*, femur; *t*, tibia; *c*, corbel; *s*, scrobe; *e*, *e*, developing tendons (flexor and extensor tibiae) and muscles; *1*, *2*, *3*, *4*, *5*, segments of the tarsus.

the claw. At pupation this tendon is drawn out to great length, the hypodermis nuclei move apart, and spend themselves completely in chitine formation. Thus is the tubular ingrowth of soft hypoderm cells transformed into a solid cord of chitine. Muscle cells developing internally at the expense of the fat are from the first intimately associated with these hypoderm cells (*cf.* Fig. 9), and through them become attached later to the tendon. Just after pupation there is a striking similarity in appearance between these tendons growing from the surface into the leg cavity and the tracheae growing from the body cavity into it.

Fig. 6 shows the leg of a young pupa within its sheath. All the leg segments are rapidly assuming their definitive form. The fourth tarsal segment has reached its maximum development; it will be relatively smaller in the adult. The broad, flat, brush-bearing pads of the third tarsal segment are here big bag-like dilatations. Corbel and scrobe are very evident upon the tibia, and the femur and other segments are full of fat, rapidly being metamorphosed into muscle.

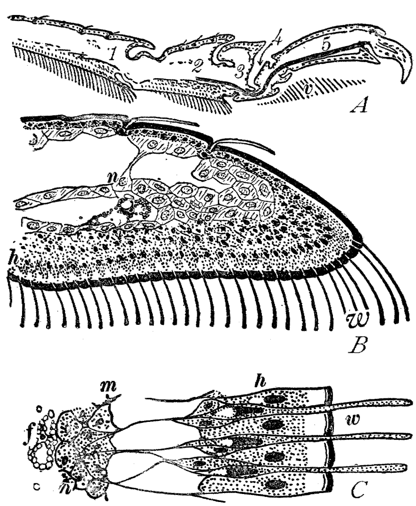

FIG. 7.—The tarsus in the pupal stage. *A*, longitudinal section of the tarsus in an old pupa. *B*, part of another section from the same series passing through one of the brushes of the 3d segment; *w*, the scales constituting the brush; *v*, the edge of one of the brushes belonging to the third segment; the tendon which retracts the claw is drawn in solid black; *n*, neuroblasts. *C*, a bit of a section through the tarsal brush in a young pupa, to show the origin of the scales; *w*, the scales; *h*, hypodermis; *m*, basement membrane; *n*, neuroblasts (undifferentiated); *f*, fat.

Fig. 7, *A*, represents the structure of the tarsus in an old pupa; externally it is practically that of the adult beetle. Fig. 7, *B*, is another section in the same series, passing through one of the lateral pads of the third tarsal segment. It shows a thinner hypodermis above, bearing scattered scales, and a thicker hypodermis below, bearing the dense tarsal brushes. Within are seen disintegrating fat cells, and other growing cells, angular and with large nuclei, which I take to be neuroblasts. Fig. 7, *C*, is from a younger pupa. It shows well the manner of development of the tarsal brush. The mother-cells of its constituent scales settle below the general level of the hypodermis; owing to close crowding, their nuclei take on a cuneate form, and on the inner side of each a minute but distinct vacuole appears. At this age the hypoderm cells generally, as here, reach their basement membrane by long, peaked internal processes. Against this basement membrane here lie heaped embryonic cells, which later differentiate as the above-mentioned neuroblasts. Subse-

quent approximation of hypoderm cells and basement membrane (due, shall we say, to the drawing in of the peaked processes ?), together with the loss of distinct cell boundaries in the hypodermis, renders the relation of parts much less clear in the later stages.

The tarsal claw and the tibial scrobe are developed alike from thick projections of hypodermis cells, forming at first a blunt point, which becomes sharp and takes on its characteristic tenacular curvature only when the chitine begins to harden. The corbel, however, being formed not at an angle of the leg, but upon an originally smooth surface, develops differently. There is a dense heaping of the hypoderm cells along what is to be the rim of the *U*-shaped corbel, among which the very large mother-cells of the fringing spines are early differentiated. Within the rim the cells are few, slender, and scattered. Outer (cuticle) and inner (basement membrane) surfaces are at first parallel; but the subsequent settling down of all the hypoderm cells upon their basement membrane leaves, where the few slender cells were within the rim, the proper concavity of the corbel.

IV. *Fat.*

The extraordinary growth taking place during the last larval stage is due almost wholly to the accumulation of fat. This occurring chiefly upon the dorsal side brings about the characteristic curvature of the larva. Hardly has growth been completed, however, before the reverse process sets in; the fat begins to be demolished and used in the construction of new parts. The external appearances accompanying the reduction of the fat have already been described. In sections the appearance is that of local disintegrations of the periphery of certain of the fat masses. Fig. 8 is a section through the middle of the thorax very near the beginning of metamorphosis. At the bases of the budding appendages and immediately above and below the alimentary canal, the fat is disintegrating. The appearance is that of the melting of frost. The fluid *residuum* flows forward into the head and laterally out into wings and legs, bearing along floating islands broken away from the fat masses.

During growth the fat is being stored in undifferentiated mesodermal cells which are free within the body cavity. These cells become greatly distended with the fat globules, which come to fill great interstices in the protoplasm toward the cell periphery. That each nucleus retains its vitality notwithstanding, is shown by its staining reactions, and by its retention of a central mass of protoplasm about itself, from which the peripheral strands that encircle the fat globules proceed. This is certainly not typical fatty degeneration; it seems to me much more properly considered to be partial anabolism, affected by these cells in their rapid elaboration of hydrocarbons during the transient period of abundant food supply. This view is corroborated by their later history. They do not (at least a majority of them do not) die with the dissolution of the fat. Nothing is plainer while one is watching the disintegration of the fat masses than that the nuclei contained therein show none of the usual signs of necrobiosis. Here and there will be seen a nucleus which, together with its enveloping coat of protoplasm, seems to be slipping itself free from its aforetime accumulation of fat. Furthermore, these nuclei thus isolated can be seen associating themselves with the developing muscle rudiments, and, apparently,

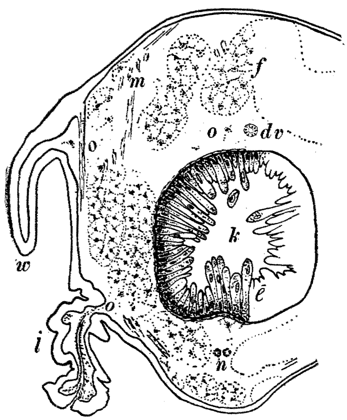

FIG. 8. — Partial cross-section of a larva, nearing pupation. *w*, fore wing; *l*, middle leg; *m*, muscles; *f*, fat; *dv*, dorsal vessel; *k*, alimentary canal; *e*, digestive epithelium, ready for dissolution; *n*, nerve cord; *o*, *o*, *o*, areas of first disintegration of the fat masses.

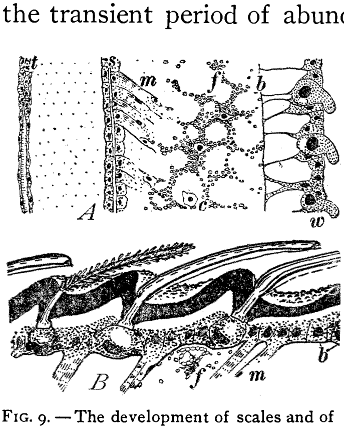

FIG. 9. — The development of scales and of muscle fibers. *A*, a bit of a longitudinal section of the femur of a young pupa; *t*, developing trachea; *s*, developing tendon (flexor tibiae); *m*, developing muscle fibers; *f*, disintegrating fat; *c*, a nucleus belonging to the fat mass isolating itself from the same; *b*, basement membrane; *w*, developing scales, in the midst of ordinary hypodermis. *B*, a bit of the body wall from a newly transformed imago, lettered as in *A*.

themselves becoming the nuclei of new muscle fibers. The single fat globules which they often carry with them and sometimes retain, even after they have become associated with the muscle rudiments, enable one to follow them easily from their former situation into this new one.

There is no destruction of any larval tissue by phagocytes during metamorphosis, but *after the imago stage has been entered upon,* large numbers of phagocytes appear in the midst of the fat along the sides of the abdomen. There are numerous embryonic or undifferentiated cells lying along the sides of the body in the larvae; and these, I believe, begin to penetrate the fat masses toward the end of the pupal stage. Fig. 10 shows the appearance they present in a recently transformed weevil. Up to this time the fat filling the abdomen has not been greatly reduced, except in the anterior end; the change of form in the abdomen in passing from larva to imago is slight as compared with that of other parts. But it is clear that the internal metamorphosis is only well under way when the external is completed. This reserve store of fat is for the completion of the still weak organs of the imago, and for nutrition during the ten long months of inactivity remaining before the flags bloom and feeding begins again.

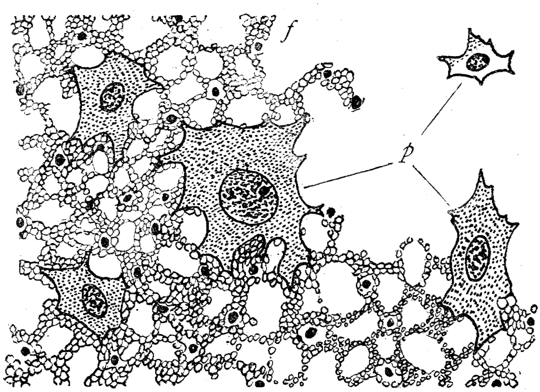

Fig. 10. — Phagocytes attacking the fat; the section is through the abdomen of a recently transformed imago. *p*, phagocytes; *f*, fat. (From a preparation made in my laboratory by Miss Elizabeth Andrews.)

After calling attention to some of the interesting features of post-embryonic development, I would not close this little paper without mentioning the exceptional availability of this species for laboratory study. On a single trip to a favorable flag clump during the latter part of July in this latitude, one may gather in

a little while enough material for studying its entire metamorphosis. This material may be excellently fixed in boiling 70 per cent alcohol, and in all stages preceding the imago the chitine is so thin as to interfere but little with section cutting.

The following conclusions from the foregoing study are believed to be new:

1. In *Mononychus vulpeculus* Fabr. there are three larval stages.

2. The full-grown larva is very degenerate, having only the merest rudiments of antennae, eyes, optic lobes, and salivary glands.

3. The greater part of the increase in size takes place in about a week after entering the third larval stage; it is due mainly to fat accumulation.

4. This brief period of feeding and rapid accumulation of half-assimilated food material is correlated with an extremely long final assimilation period, lasting through months of imaginal life.

5. There is no real invagination of the buds of wings or legs.

6. Many nuclei of fat cells persist after the dissolution of the fat masses, free themselves from these masses, retaining about themselves an investment of protoplasm, associate themselves with developing muscle fibers, and, probably, themselves become the nuclei of new muscle fibers.

7. Phagocytosis, which was observed only in the fat masses along the sides of the abdomen, occurs only after external metamorphosis is complete.